NanoSIMS reveals activity in deepest samples ever collected by marine scientific drilling

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Advances in marine scientific drilling and microbiological methods have led to the discovery of seemingly ubiquitous microbial life in a range of deep biosphere habitats. To determine a possible depth limit of life, IODP Expedition 337 successfully recovered core down to a record-breaking 2.5 km below seafloor from a deeply buried coalbed system of terrestrial origin and low thermal alteration (lignite). Initial results showed cell abundances no longer tracked the expected depth trend recovered from all previous drilling operations, suggesting life-limiting conditions had been reached (Inagaki et al., *Science*, 2015). The coalbed community, however, provided an exception with higher (10-100X) cell concentrations than the surrounding shale and sandstone lithologies. C1 metabolisms, particularly those associated with methoxy groups or low molecular weight organic acid byproducts of coal breakdown, may serve as a potential source of carbon for this coalbed deep biosphere community. To determine general cell activity (deuterated water) and nitrogen and C1-carbon assimilation strategies, 2.5 year-long Stable Isotope Probing (SIP) incubations were interrogated by NanoSIMS. We found an active coalbed microbial community and provide constraints on doubling times, growth patterns and C1-carbon metabolic capabilities in this unique environment.